Applicant: Wei-Yu Lo et al. Attorney's Docket No.: 12875-002001 / 0643-5299US

Serial No.: 09/778,516 Filed: February 7, 2001

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## Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

## Listing of Claims:

- 1. (Previously presented) A Lac shuttle vector, comprising:
- (a) a region which regulates a plasmid copy number, wherein said region comprises an E. coli replication origin sequence;
- (b) a eukaryotic gene expression cassette, which comprises a eukaryotic gene transcriptional promoter sequence, a multiple cloning site and a transcriptional terminator sequence, wherein a desired gene is inserted into said multiple cloning site;
- (c) a lactic acid bacterial plasmid sequence, which comprises a plus origin of replication, and a nucleic acid sequence encoding a protein which is involved in replication of the lactic acid bacterial plasmid; and
- (d) a marker gene that is not an antibiotic resistance gene and is operably linked to a promoter sequence.
- 2. (Original) The Lac shuttle vector as claimed in claim 1, wherein said eukaryotic gene transcriptional promoter is cytomegalovirus (CMV) promoter.
- 3. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein said lactic acid bacterial plasmid sequence is the plasmid of 2.1 kb in size isolated from Lactobacillus plantarum.
- 4. (Previously presented) The Lac shuttle vector as claimed in claim 3, wherein the protein which is involved in the lactic acid bacterial plasmid replication is Rep A protein consisting essentially of 317 amino acids.

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5. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein said marker gene is β-galactosidase gene.

- 6. (Original) The Lac shuttle vector as claimed in claim 5, wherein the promoter of said β-galactosidase gene is erythromycin resistance gene promoter.
- 7. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the Lac Shuttle vector comprises the nucleotide sequence set forth in SEQ ID NO:1 or a complementary nucleotide sequence thereto, or a degenerate variant thereof that contains degenerative protein-coding sequences.
- 8. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the Lac Shuttle vector comprises the nucleotide sequence set forth in SEQ ID NO:2 or a complementary nucleotide sequence thereto, or a degenerate variant thereof that contains degenerative protein-coding sequences.
- 9. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the Lac shuttle vector is selected from the group consisting of:
- (a) pCLP7 having the configuration of restriction sites in FIG. 4, American Type Culture Collection Accession No. PTA-2661; and
- (b) pCLP8 having the configuration of restriction sites in FIG. 4, American Type Culture Collection Accession No. PTA-2663.
- 10. (Previously presented) The Lac shuttle vector as claimed in claim 1, wherein the vector is for transforming a host cell, the host cell being a Gram-positive bacterium, and the endogenous β-galactosidase gene of the host cell being non-functional.

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11. (Previously presented) The Lac shuttle vector as claimed in claim 10, wherein the host cell is the Lac- mutant of *Lactobacillus casei*, subsp. casei, which is designated Ana-1, American Type Culture Collection Accession No. PTA-2662.

- 12. (Previously presented) A kit for expression of a gene, comprising:
- (a) the Lac shuttle vector as claimed in claim 1;
- (b) a host cell in which the endogenous  $\beta$ -galactosidase gene thereof is non-functional; and
  - (c) a eukaryotic cell.
- 13. (Previously presented) A DNA vaccine composition comprising a Lac shuttle vector that contains:
- (a) a region which regulates a plasmid copy number, wherein said region comprises an E. coli replication origin sequence;
- (b) a eukaryotic gene expression cassette, which comprises a eukaryotic gene transcriptional promoter sequence, a multiple cloning site and a transcriptional terminator sequence, wherein an antigenic gene is inserted into said multiple cloning site;
- (c) a lactic acid bacterial plasmid sequence, which comprises a plus origin of replication, and a nucleic acid sequence encoding a protein which is involved in replication of the lactic acid bacterial plasmid; and
- (d) a marker gene that is not an antibiotic resistance gene and is operably linked to a promoter sequence.
- 14. (Previously presented) A method for selection of a host cell containing a vector, comprising:
- (i) introducing into said host cell the Lac shuttle vector as claimed in claim 1, wherein the endogenous β-galactosidase gene of said host cell is non-functional; and
- (ii) culturing said host cell transformed in step (i) under conditions in which lactose is the only carbon source, thereby selecting a host cell comprising the Lac shuttle vector of claim 1.